A PROGRAM IN GENETICS AND MOLECULAR BIOLOGY

Genetics Department

Stanford University School of Medicine

October 1968 - October 1973

Submitted by:

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Department of Genetics
Stanford University School of Medicine
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This application is intended as a continuation of Grant Number GB 4430 (expiring 10/15/68) entitled "A Program in Genetics and Molecular Biology".

A PROGRAM IN GENETICS AND MOLECULAR BIOLOGY

The development and expansion of the Genetics Department in its new facilities in the Clinical Sciences Research Building of the Stanford University School of Medicine, occupied in March 1966, has been greatly aided by funding from the current "Program in Genetics and Molecular Biology" -- National Science Foundation Grant No. GB 4430. The main emphasis of this grant has been the provision of major items of equipment whose use is commonly shared by all members of the Department. The greater flexibility which has derived from this type of departmental grant compared to the individual research grants has enabled us to operate much more efficiently and to respond rapidly to equipment needs as research programs change and develop. The success of the program as an essential complement to individual funding can be judged from the variety of advances documented in the departmental bibliography. As the program has continued, and many, if not all, of the equipment needs have been met, the Department has been evolving other cooperative efforts to provide for the maximum and efficient utilization of our facilities. It has become increasingly clear from this that there is a considerable need for personnel and services which operate at a departmental rather than individual level and it is mainly for their provision that the present continuation application is made. Many areas of our individual research programs converge on common techniques and facilities, yet often no one individual is in a position to justify the entire use of a Professional Assistant or laboratory facilities for these particular needs. The operation of the departmental amino acid analyzer purchased from funds of the present

Program is an excellent example. Again the ordering and disposal of supplies and the day by day maintenance of equipment and common wash-up and sterilization facilities is most effectively accomplished by a Departmental Laboratory Coordinator.

The faculty group participating in this application consists of:

Professor J. Lederberg, Executive - Genetic chemistry of bacteria. Computer system for biochemical analysis.

Professor W. Bodmer - Genetic chemistry of transformation. Somatic cell genetics and genetics of human white cell antigens. Population genetics.

Professor E. Shooter - Molecular neurobiology. Chemical ontogeny and polymorphism of nervous system proteins. Nerve growth factor proteins.

Associate Professor L. Herzenberg - Immunogenetics and somatic cell genetics.

Assistant Professor A. Ganesan - Mechanisms of genetic recombination. In vitro synthesis of transforming DNA.

Senior Research Associate E. Levinthal - Instrumentation research.

Senior Research Associate B. Halpern - Reagents and techniques for ultramicroanalysis.

In addition, a number of research associates of senior stature are connected with these and other programs, including Dr. S. Liebes (physics of mass spectrometry). The substantial engineering developmental program in automated instrumentation under N.A.S.A. auspices continues to converge with the main research areas of the department, the development of an automated cell separator being one excellent example of the collaboration.

The Exchange Program in Genetics and Molecular Biology between Stanford University and the University of Pavia, funded in part by NSF, is also now in operation and the following Pavia faculty and students are, or will shortly be, working in the Genetics Department: Professor L. Cavalli-Sforza, Drs. M. Polsinelli, S. Barlatti and A. Cefferi and P. Pignatti.

Professors E. Glassman of the University of North Carolina and Sarane T. Bowen of San Francisco State College will be spending their sabbatical leaves in the department in 1968-69.

A detailed budget for the first year of the program is attached, as well as a general breakdown of expenditures for the subsequent second, third, fourth and fifth years.

NATIONAL SCIENCE FOUNDATION

Washington, D. C. 20550

RESEARCH GRANT BUDGET SUMMARY

Institution:	Principal Investigator:	Program Name:	Duration:	Grant Number:
Stanford University School of Medicine	Joshua Lederberg Genetics Department	A Program in Genetics and Molecular Biology	10/15/68 to 10/15/69	

	NSF Funded Man Months (Cal)	Grantee Man Months (Cal)	NSF Grant	Grantee Share
A. SALARIES AND WAGES				
1. Senior Personnel				
a. Principal Investigator				
Joshua Lederberg	1			3,369
b. Faculty Associates				
W. Bodmer, A. Ganesan, L. Herzenberg, E. Levinthal, E. Shooter				
2. Other Personnel: Technical				
a. Administrative Officer				
D. Stuedeman	4		4,000	
b. Laboratory Coordinator				
M. Thomas	12		7,600	
c. Professional Asst. to operate Dept. Amino Acid Analyzer				
V. MacPhee	12		8,400	
d. Professional Asst. to operate Dept. Tissue Culture Lab	12		8,400	
e. Lab. Technician for Dept. Animal House				
F. Rodriquez	12		6,600	
f. Machinist	2		2,000	
Total			37,000	3,369
B. FRINGE BENEFITS (11.6%)			4,292	391
Total			41,292	3,760
C. PERMANENT EQUIPMENT				
1. Large liquid N_2 tank			5,000	
2. Spinner culture apparatus			5,000	
Total			10,000	
D. EXPENDABLE SUPPLIES AND EQUIPMENT				
Materials for use in departmental facilities, e.g. wash up room and sterilization, Media Room, Animal Room; chemical and glassware for departmental stores; reference materials and library			7,000	

RESEARCH GRANT BUDGET SUMMARY (continued)

	NSF Grant	Grantee Share
E. OTHER DIRECT COSTS		
Maintenance on equipment (purchased on previous grant)	2,000	
Minor laboratory rearrangements	2,000	
Rent of two complete terminals for use with ACME	4,500	
Computer time for batch processing on campus computer facility at $400/hr.	2,000	
Total	10,500	
F. TOTAL DIRECT COSTS	$ 68,792	3,760
G. INDIRECT COSTS (57% S&W)	21,090	1,920
H. TOTAL COSTS	89,882	5,680
REQUESTED FROM NSF	89,882	

Stanford University
School of Medicine

A Program in Genetics
and Molecular Biology

BUDGET FOR 2ND AND SUBSEQUENT YEARS -

	2nd	3rd	4th	5th
PERSONNEL	43,300	45,465	47,738	50,125
EQUIPMENT	10,000	10,000	10,500	10,500
EXPENDABLE SUPPLIES	7,000	7,300	7,600	7,900
OTHER	10,500	11,000	11,500	12,000
DIRECT COSTS	70,800	73,765	77,338	80,525

The figures for Personnel have been increased by 5% each year.

Justification for Budget

A. Personnel

As the research programs of the various faculty members have developed and expanded it has become clear that they overlap in many areas, e.g. in protein sequence work and tissue culture, and we have therefore set up or are setting up such facilities on a departmental rather than individual basis. This provides for the most efficient utilization of facilities and equipment and also, in instances where a person's needs are relatively small (but vital) and do not justify individual requests for funding, for the greatest flexibility in meeting specific research needs. The department has operated an amino acid analyzer on this basis with great success for the past nine months and the budget therefore requests continuing stipend for a Professional Assistant to run this instrument.

Three faculty members at the present use tissue culture and the operations again are on a scale that coordination of equipment and media would be of great benefit. It is envisaged that a Professional Assistant would be in charge of such a coordinated effort and that this person would have also sufficient capability to assist each of the faculty groups as required. The use of the Genetics Department Animal Facility has increased far beyond the load for one person and a second Animal Room technician was recently hired. In that much of his work is for general members of the Department rather than one Faculty member, his stipend is now included in the departmental application.

Two positions are listed for a part salary for the Senior Laboratory Coordinator and full time salary for his assistant. Between them they take

care of the routine management of the departmental facilities and its technical staff, the organization of the wash and sterilization facility, much of the ordering of supplies and equipment and any research that this may involve, and generally the day to day interactions with the service engineers of the building.

A continuing part time salary for a machinist is also included in this application. His services in repairing and modifying equipment and installing minor laboratory fittings continue to be invaluable.

C. Equipment

This category is now much reduced in level compared to the current grant and will be used as indicated to buy, again, equipment for departmental use, for example, for cell storage and culture.

D. Expendable Equipment and Supplies

These items include materials for use in the common wash up and sterilization facility, the animal house and for departmental stocks of glassware, scintillation vials and chemicals and solvents used in relatively large amounts. Also it includes funds for the purchase of necessary reference materials (e.g. Automatic Subject Citation Alert, ASCA) and other library supplies.

E. Other Direct Costs

With the availability of ACME (Advanced Computer Facilities for Medical Education) this Department makes large scale and effective use of these major facilities. The cost of the rent of two of our ACME terminals is included in this category as is also computer time for the overnight batch processing of some of our larger requirements.

Existing Facilities

The department covers some 11,000 square feet in the new Clinical Sciences

Building of the Stanford Medical Center complex providing integrated areas for microbial, cell and human genetics, molecular neurobiology and computer work. Common facilities for wash up and sterilization are available as are hot and cold room facilities. The Instrumentation Research Laboratories are housed in 6,000 sq. feet of appropriately designed areas on the Ground Floor beneath the Genetics Department.

The department is equipped with all the usual apparatus for work in the fields outlined above. Major items of equipment include:

Tricarb scintillation spectrometer

Spinco analytical ultracentrifuge

Spinco and IEC preparative centrifuges

Spinco amino acid analyzer

Zeiss spectrophotometer

Bendix and Quadripole mass spectrometer with interfaces to the IBM 360/50 computer

Existing Funding. No other funding matches the purpose of this application, however, component elements of our investigations are supported as follows:

From National Institutes of Health

Grant No.	Grant Title	Dates	Direct Costs
AI-5160	Genetics of Bacteria	9/1/64-8/31/68	$ 253,000
GM-14108	DNA Synthesis & Genetic Recombination	6/1/66-5/31/69	104,300
GM-14650	Genetics of Human Tissue Antigens	12/1/66-11/30/69	124,000
NB-04270	Molecular Neurobiology	12/1/67-11/30/70	202,000
GM-12075	Genetics of Immunoglobulins	6/1/64-5/31/69	149,500
CA-04681	Genetic Studies with Mammalian Cells	9/1/67-8/31/72	272,000
GM-295-10	Final year of current period of Training Program	7/1/68-6/30/69	115,000
Application Pending	Renewal of Training Program in Genetics	7/1/69-6/30/74	756,650
GM-35002	Career Award: Dr. Walter F. Bodmer	1/1/67-12/31/71	120,000

From National Science Foundation

GB 5862	Genetic Chemistry of DNA Mediated Bacterial Transformation	12/1/66-11/30/68	76,950
GB 6878	Subunit Structure of Nerve Growth Factor	9/1/67-8/31/69	44,500

From National Aeronautics and Space Administration

NASA NsG 81-60	Cytochemical Studies of Planetary Microorganisms (Instrumentation Research Lab.)	9/1/67-8/31/68	410,000

Joshua Lederberg - Genetics of Bacteria

The DNA transfer system ("transformation") in Bacillus subtilis offers a particularly favorable opportunity to study the physical and chemical properties of DNA molecules in relation to their biological activity. Over the past several years my principal co-investigators have been Professor Walter F. Bodmer and Professor A. T. Ganesan. Their work now has become well differentiated and receives independent support, but the studies summarized in the attached publications were initiated during the current term of the grant.

The main questions we have examined have concerned:

The linkage system, as manifested in DNA transfer;

The fractionation of biologically active DNA, assigning different genetic activities to different average base compositions;

The effects of fragmentation of DNA molecules by physical shearing, and by nuclease attack;

The mechanism of integration of DNA in the course of the transformation process;

The association of the DNA replication process with a cell-membrane bound polymerase.

In collaboration with Professor Arthur Kornberg and other members of the Department of Biochemistry we had also attempted to demonstrate the replication of biologically active DNA by E. coli DNA polymerase. Those experiments were, however, unsuccessful -- in considerable part, as we now know, because of our ignorance of polynucleotide ligase.

The main impact of these studies has been to verify the correspondence of the properties of genetic activity of DNA with distinctive sequences of nucleotides, and to provide some further technical facilities for the ultimate description of genetic information in chemical terms. During the period covered by this report many workers have entered similar fields and the literature now contains many examples of the building of new edifices on previously established foundations.

I propose now to concentrate on the natural or experimentally induced occurrence of insertions and inversions in B. subtilis. In enteric bacteria, mapping studies have shown a remarkable congruence of gene sequences among bacterial species which have already diverged significantly in DNA homology as tested by hybridization-reanealing experiments. Conservation of gene order is also evident in the organization and persistence of operons, showing coordinate repression; these are frequent in bacteria but rare in higher forms. Most or all of the examples of inversions found experimentally in bacteria can be explained either as methodological artifacts (1) or as consequences by crossing over of episome-bound segments with redundantly homologous segments of the whole DNA (2).

These observations were vague forerunners of the now accepted understanding that bacteria differ from eukaryons in 1) The simple organization of the chromosome as a single polynucleotide sequence, and 2) The absence of any common mechanism for "holo-ligation repair" of the chromosome. The recent clarification of the dark repair of single-strand breaks in DNA (reviewed in 3) has pointed up mechanisms of template-directed repair (hemiligation) in bacteria. These are fundamental to recovery from UV-damage, to recombination mechanisms, and probably to the post-editing of newly replicated DNA. There is, on the other hand, no good evidence for any normal mechanisms for joining or rejoining broken DNA strands except for hemi-ligation, i.e., when the broken strand is entwined with a complementary template strand that spans the broken ends.

It may be previous to define a process that may not exist, but we are then looking for holo-ligation, i.e., some way to join broken strands, or double helices, without the help of a homologous template. The potential extent and limitations of holo-ligation relate to many current problems in chemical genetics and cell biology. These include, for example:

1) Genetic variation in recovery from X-ray damage and the mechanism of recovery from double-strand scissions.

2) A sharper confrontation with chromosome structure of eukaryons in which broken chromosomes can indeed be rejoined (but is this a covalent nucleotide assembly?)

3) Transcription problems: framing, the reading of inverted sequences, and strand selection.

4) The barrier to promiscuous recombination of DNA from different sources.

Point 4 may be the most far-reaching for every level of genetic manipulation, both investigative and applied. The rejection of foreign DNA by competent Bacillus subtilis cells may illustrate one evolved mechanism whereby a cell has been able to protect itself against unlimited intrusion by foreign genes, arising by chemical scrambling or by virus infection. The research utility of freely moving genes from another species into bacteria needs no elaboration, e.g. for the study of transcription-control, and to facilitate analyzing the genetic competence of DNA from differentiated tissues of higher organisms.

Important practical utilities would follow from the incorporation of human genes into suitable cryptic virus DNA for kind of transductional therapy for human genetic disease (4).

References:

1. Glansdorff, N., 1967. Pseudoinversions in the chromosome of Escherichia coli K-12. Genetics 55:49-61.

2. Berg, Claire M. and Roy Curtiss III, 1967. Transposition derivatives of an Hfr strain of Escherichia coli K-12. Genetics 56:503-525.

3. Hanawalt, Philip C., 1968. Cellular recovery from photochemical damage. Ch. 11, Photophysiology, Vol. III (A. C. Giese, ed.) Academic Press, New York.

4. Rogers, Stanfield, 1966. Shope papilloma virus: A passenger in man and its significance to the potential control of the host genome. Nature 212:1220-1222.

Personnel

Postdoctoral:

S. Barlati	Polysome aggregation in B. subtilis
M. Polsinelli	Genetic translocation in B. subtilis

Predoctoral:

H. Eisenstark	Conditional lethals in B. subtilis; toxicity of DMSO
I. Majerfeld	Adding homopolymer terminations to B. subtilis DNA
W. Spiegelman	Regulation of lambda bacteriophage
L. Okun	High molecular weight transforming DNA

JOSHUA LEDERBERG

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Phone:
(415) 321-1200 Ext. 5801

Education:

1938-41	Stuyvesant High School (New York City)
1941-44	B.A., Columbia College
1944-46	Enrolled as medical student, Columbia University College of Physicians and Surgeons
1946-47	Ph.D., Yale University. Sc.D. (h.c.) 1960; also Wisconsin (1967); Columbia (1967).

Experience:

1945-46	Research assistant in zoology (with Professor F. J. Ryan), Columbia University
1946-47	Research fellow of the Jane Coffin Childs Fund for Medical Research at Yale University (with Professor E. L. Tatum)
1947-59	Professor of Genetics, University of Wisconsin
1950	Visiting Professor of Bacteriology, University of California, Berkeley
1957	Fulbright Visiting Professor of Bacteriology, Melbourne University, Australia
1957-59	Chairman, Department of Medical Genetics, University of Wisconsin
1959-	Professor, Genetics and Biology, and Executive Head, Department of Genetics, Stanford University
1961-	Director, Kennedy Laboratories for Molecular Medicine, Stanford University

Special field: Genetics, chemistry and evolution of unicellular organisms and of man.

Distinctions:

1957	National Academy of Sciences
1958	Nobel Prize in medicine (for studies on organization of the genetic material in bacteria)

Public responsibilities:

1961-62	President (Kennedy)'s Panel on Mental Retardation
1950-..	President's Science Advisory Committee panels. National Institutes of Health, National Science Foundation study sections (genetics)
1958-..	National Academy of Sciences: committees on space biology
1960-..	NASA committees; Lunar and Planetary Missions Board
1967-	NIMH: National Mental Health Advisory Council

Personal Data:

b. May 23, 1925; Montclair, New Jersey

Walter F. Bodmer - Genetic Chemistry of DNA Mediated Bacterial Transformation; Somatic Cell Genetics and the Genetics of Human White Cell Antigens; Population Genetics.

1. Genetic Chemistry of DNA Mediated Bacterial Transformation

The main aim of this research is to further the understanding of the processes of integration and recombination during DNA mediated transformation in Bacillus subtilis. Earlier work from our laboratory was instrumental in showing that donor DNA was incorporated into small single-stranded regions of the recipient genome (Bodmer and Ganesan 1964) and in establishing the extent to which DNA synthesis may be involved during transformation (Bodmer 1965). This work clearly showed that there is no major amount of DNA synthesis during uptake and integration of donor DNA, though the amount of repair synthesis that may be involved and the specific enzymatic steps leading to final integration remain incompletely understood. Understanding these problems remains a major part of our research effort. Specific research projects currently underway or being planned are

a) The properties of a mutant which is a genetic rearrangement will be investigated during transformation, with special reference to testing for heterozygosity in the products of transformation.

b) Attempts are being made to assess the true extent of repair synthesis during transformation. High specific activity P^{32} labelling during transformation and 5-Bromouracil labelling during transformation accompanied by physical separation of competent cells, are the two techniques currently envisaged for these studies.

c) A double marker transformation assay has been shown to be a likely indicator of "cross-correction repair" during transformation. The

effect of UV sensitive mutants on this system are being investigated.

d) work with multiply marked recipient strains is continuing with a view to determining further the relationship between DNA integration during transformation and regions of initiation of DNA synthesis. Experiments are also planned to test whether protein synthesis is required for reinitiation of DNA synthesis following transformation.

e) Further experiments are planned on the early events following uptake of donor DNA during transformation, in particular to determine the nature of the early native material found following addition of donor DNA. In addition, efforts will be made to characterize the transition from non-covalent to covalent association between donor and recipient DNAs.

2. Human Somatic Cell Genetics and the Genetics of Human White Blood Cell Antigens.

a) Our laboratory,in collaboration with Dr. Rose Payne of the Department of Medicine, has been instrumental in identifying some of the major antigens of the HL-A human white blood cell antigen polymorphism and in interpreting the relationships between the genetic determinants for these antigens (Payne et al 1964, Bodmer and Payne 1965, and Bodmer et al. 1966). During the last 18 months we have developed a convenient microcytotoxicity assay combining Terasaki's micro droplet assay with the technique of fluorochromasia (Bodmer et al 1968). We have used this assay extensively for population genetic and other studies directed towards further development of knowledge of the genetics of the human white cell antigen systems. Current areas of investigation are

i. We are attempting to devise more sensitive assays for the antigens using anti-human gamma-globulin serum both for cytotoxic and agglutination assays.

ii. New antigenic specificities are being characterized by absorption analysis of a number of sera, combined with population and family studies.

iii. We have undertaken a major population genetic study, in collaboration with Professor L. L. Cavalli-Sforza, of the pygmies of Central Africa and of other African populations. We plan to extend these population studies to other racial groups through collaboration with other investigators.

iv. We are investigating the potential uses of cattle *isoantisera* for typing human white cell antigens and the general question of species cross-reactivity with respect to these antigenic systems.

b) During the last year we have initiated a program of research in human somatic cell genetics. This is based on using the technique of cell fusion mediated by inactivated Sendai virus. Hybrid lines are being made by fusing human peripheral blood lymphocytes with base analogue resistant mouse cell lines derived from known inbred mouse strains. Hybrids are initially recognized by their chromosome constitution. A major source of genetic markers are the white cell antigens being studied in our laboratory. A micro-mixed agglutination technique for the identification of these antigens on cell cultures is being developed and is being applied to study the distribution of these antigens on our human-mouse hybrids. Other genetic markers will, of course, be

studied, in particular electrophoretically distinguishable variant enzymes. The karyotypic and genetic constitution of hybrid lines is being studied and attempts are being made to correlate the presence of combinations of human genetic markers with the chromosomal constitutions of the hybrids. These studies will be coupled with attempts to obtain control changes in the hybrid karyotype. Other lines of human cells will be used for studies related to the problem of gene expression as a function of differentiated cell type.

3. Population Genetics

The emphasis of work in this area is on the theoretical analysis of population genetic models and their interpretation, particularly with relation to the problems of human genetics. For some years I have been involved in an active program of research in theoretical population genetics with Professor Karlin of the Mathematics Department. This unique relationship has provided an opportunity for combining a sophisticated mathematical approach with direct contact with experimental genetics. Recently, work has been done to extend understanding of models for the Rhesus blood group incompatibility system. In collaboration with Professor L. L. Cavalli-Sforza, new models have been developed to explain observed differences in gene frequency in terms of observed migration patterns, using a migration matrix approach. Further work is planned on the problem of the interaction of linkage and selection, particularly with respect to the conditions under which there is selection pressure for tighter linkage and the problems of dealing with more than two loci. In addition, some work has been done on using data on amino acid substitutions to match observed evolutionary rates with those expected from population

Walter F. Bodmer - Genetic Chemistry of DNA Mediated Bacterial Transformation; Somatic Cell Genetics and the Genetics of Human White Cell Antigens; Population Genetics.

genetic theory. A major survey of theoretical population genetics, undertaken in collaboration with Professor Karlin and Mark Feldman, a graduate student, is nearing completion.

Personnel

Postdoctoral

A. J. Darlington	Genetic chemistry of DNA mediated bacterial transformation
V. Miggiano	Human somatic cell genetics
T. Iha	Human white cell antigen genetics
F. Scudo	Theoretical population genetics

Predoctoral

M. Nabholz	Human somatic cell genetics

Research Assistants

L. Wang	Genetic chemistry of DNA mediated bacterial transformation
J. Bodmer	Genetics of human white cell antigens
G. Gerbrandt	" " " " " "
M. Tripp	" " " " " "

WALTER F. BODMER

CURRICULUM VITAE

Personal Data:

Born: Frankfurt am Main, Germany, January 10, 1936
British citizen, U.S. Immigrant
Wife: Julia Gwynaeth Bodmer
3 children
Soc.Sec. No. [redacted]

Education:

1953-56	B.A., Clare College, Cambridge University
1956-59	Ph.D., Clare College, Cambridge University

Experience:

1958-60	Research Fellow, Clare College, Cambridge
1960-61	Demonstrator, Department of Genetics, Cambridge University
1961	Official Fellow, Clare College, Cambridge
1961-62	Fellow, Visiting Assistant Professor, Department of Genetics Stanford University School of Medicine.
1962-66	Assistant Professor, Department of Genetics, Stanford University School of Medicine.
1966-68	Associate Professor, Department of Genetics, Stanford University School of Medicine.
1968-	Professor, Department of Genetics, Stanford University School of Medicine.

Special Field: Chemical Genetics, Human and Population Genetics.

Public Responsibilities:

1964-67	National Science Foundation, Genetics Panel
1964-67	National Institute of Allergy and Infectious Diseases, Committee for Collaborative Research in Transplantation and Immunology
1968-	Associate Editor, American Journal Human Genetics

A. T. Ganesan - *In vitro* Synthesis of Transforming DNA, Mechanism of Genetic Recombination

The *Bacillus subtilis* genome replicates sequentially from a fixed origin, as judged by isotopic transfer and gene frequency distributions in transformation experiments. An *in vitro* system using *E. coli* polymerase for the replication of DNA from *B. subtilis* yielded products which were biologically inactive. Thermal denaturation of these molecules was spontaneously reversible. These abnormalities may reflect interruptions and lack of control in coherent replication in the *in vitro* system.

Earlier experiments in our laboratory suggested that *in vivo* the nascent DNA might be bound to a particulate fraction which also contained DNA polymerase of high specific activity. Using the nascent DNA already present, the polymerase in the particulate fraction was able to synthesize DNA (Judged by the incorporation of the labeled deoxytriphosphates into cold acid precipitable material) when supplied with all four deoxytriphosphates and Mg^{++}. Our approach to the problem of synthesizing biologically active DNA *in vitro* has been to study the pattern of replication by this particulate fraction, starting from relatively crude preparations and following it through different steps of purification of the polymerase.

Careful isolation of the active protein complex free of cell membrane and other components has resulted in a purification of 100 to 150 fold compared to the initial lysate. This preparation has been found to differ in some respects from the highly purified preparations of DNA polymerase from *E. coli* and *B. subtilis*. Unlike the latter, our preparation preferred native bihelical DNA to denatured DNA as a primer. Double stranded DNA was 6 to 8 times more efficient as primer than denatured DNA. In our system dAT-copolymer

A. T. Ganesan - *In vitro* Synthesis of Transforming DNA, Mechanism of Genetic Recombination. (continued)

was only 2 to 4 times more active as a primer than the native DNA, while with the purified polymerases dAT-copolymer has been reported to be 20 times more active.

The enzyme complex sediments with a unimodal distribution in sucrose gradients. Of the ions tested Mg^{++} was the most effective in the reaction. Traces of Na^{+} and K^{+} stimulated the reaction in the presence of Mg^{++}. In a reaction, 20% of the amount of primer added was synthesized in 30 minutes. There was no significant amount of exonuclease activity found in the polymerase. The endonuclease activity associated with the preparation can be partially inhibited by RNA.

When transforming DNA labelled with N^{15}, deuterium and H^{3} was isolated from genetically marked *B. subtilis* and used as a primer in a reaction of our partially purified preparation with light (N^{14}, H) C^{14} labelled deoxytriphosphates, it was possible to demonstrate synthesis of DNA molecules of lighter density as observed by CsCl density gradient centrifugation. 80% of the molecules are denaturable and 90% are rendered into acid soluble mononucleotides by *E. coli* Exonuclease-1. The products composed of hybrid molecules containing one strand of heavy DNA and one strand of light DNA. The hybrid molecules are biologically active. In addition to the hybrid, 4% of the biological activity was associated with completely light and hybrid. Of these light molecules, at least 10% carry 3 known genetically linked genes, while the majority are only active for single gene transformation. The enzyme complex is presently studied by different physical techniques, to detect various activities, that form the active complex.

A. T. Ganesan - *In vitro* Synthesis of Transforming DNA, Mechanism of Genetic Recombination

Personnel

Postdoctoral

F. Gillin	Genetic control of DNA Synthesis in *B. Subtilis*

Predoctoral

P. Laipis	*In vitro* synthesis of transforming DNA

Research Assistant

N. Buckman	Mechanism of genetic recombination *In vitro* synthesis of transforming DNA